IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 9870
Dale B. Schenk	Examiner: Anne Marie Sabrina Wehbe
Application No.: 09/723,713	Technology Center/Art Unit: 1632
Filed: November 27, 2000	Declaration of Dr. Weiner under 37 CFR 1.132
For: PREVENTION AND TREATMENT OF AMYLOIDOGENIC DISEASE	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, David Weiner, state as follows:

1. My current position is Program Chair, Gene Therapy and Vaccines, Associate Professor, Department of Pathology and Laboratory Medicine, University of Pennsylvania College of Medicine. I have a B.S. from Stony Brook, NY and M.S. and Ph.D. degrees from University of Cincinnati.

2. I am a founder of the field of DNA vaccines, and have actively conducted research in this field for almost 20 years. My group and collaborators were responsible for the first DNA vaccine investigational new Drug applications (IND's). These IND's were for HIV immune therapy. We have vaccinated over 160 individuals with HIV-1 DNA vaccines. These studies established that DNA vaccines are well tolerated and immunogenic in humans. Subsequently, we have developed a new set of more potent second generation DNA which have exhibited improved potency in non human primates. These vaccines are now in clinical trials for HIV prophylaxis as well as immune therapy. I am on the executive board of the American Society of Gene Therapy, I am the meeting organizer of the bi Annual international DNA vaccines meeting,

I am an advisor to the WHO on regulatory aspects of DNA vaccines, and the US FDA on DNA vaccines, I regularly read the scientific literature relating to gene therapy and review for all of the major Gene Therapy journals, and I give seminars and regularly attend meetings and seminars in this field. I have also been a consultant to Wyeth for several years, which I understand is a licensee of the above-captioned application.

3. I am already generally familiar with the work described in the patent application through my role as a consultant to Wyeth. However, for purposes of this declaration, I have reviewed the above application, and pertinent official actions and responses in the file history. I understand that the above application was filed in May of 1999 and should be evaluated as of the state of the art of that date.

4. The Examiner's comments make it appear as if gene therapy was effectively moribund in 1999, and there was virtually no chance of successfully implementing a clinical therapy at that date without further developments in the field. Although I would agree there have been occasional setbacks however, many advances are being made. For example this year two DNA vaccine products were licensed, both in the field of veterinary medicine, furthermore the gene therapy vector based approaches of Adenovirus vaccines and DNA vaccines for HIV have entered in large phase II trials sponsored by a highly serious and highly esteemed groups of investigators at the NIH Vaccine research center and at the pharmaceutical company Merck. Obviously both groups only move such an approach forward if they believe that these are the most promising of the arsenals for development. I believe that as a whole the Examiner portrays an unduly negative picture. In every year since 1989, numerous human clinical trials have been initiated. For example, 37 human clinical trials were initiated in 1993, 38 in 194, 67 in 1995, 51 in 1996, 82 in 1997, 67 in 1998, 117 in 1999, 96 in 2000, 104 in 2001, 82 in 2002, 76 in 2003 and 86 in 2004. These trials included a variety of delivery techniques including retroviruses, adenovirus, naked DNA and adenoassociated virus and a variety of conditions to be treated. Before a drug can enter human clinical trials, the investigator must submit an application to the FDA (or similar body in other countries) detailing preclinical work and providing an explanation why the investigation may be successful. This explanation must be found convincing to the FDA before the trial can be allowed to proceed. The numbers of approved clinical trials show that

gene therapy was regarded as sufficiently mature for substantial use on human patients by at least the mid 1990's and has remained so ever since. In fact, gene therapy for vaccines and immune therapy approaches is believed by many to be among the most promising of all therapeutic fields.

5. I acknowledge that gene therapy has at times received negative press, a notorious example being associated with a clinical trial to treat children with severed combined immuno deficiency disease (SCID) due to a defective gene encoding the enzyme adenosine deaminase in the X-chromosome. In the absence of treatment, these children must be kept in containment bubbles and usually die at a young age. The clinical trial used retroviral vectors to replace the defective gene in the children. The trial was highly successful in treating the underlying defect in the children. Children, for the first time since their infancy, could leave their containment "prisons" hug their parents and siblings, play with friends, go to school, in other words live completely as children should. However, it was subsequently found that after several years about 10% of the treated children developed leukemia. Notwithstanding that the leukemia was itself treatable in most children and the children would have died without the gene therapy, the trial was halted by the regulatory body, which is waiting for non retroviral vectors for gene therapy delivery such as the new lentiviral vectors that are under development. It is widely believed that these new vectors will successfully treat this disease with out the associated leukemia risk. Although this end to the trial brought a lot of bad publicity for gene therapy, most considered the trial on the whole to have been a success in demonstrating that gene therapy could achieve a substantial clinical benefit in one of its more difficult applications (i.e., gene replacement therapy).

6. As I have indicated gene therapy has been used for treatment of a wide variety of conditions. The degree of difficulty depends on the condition. For example, treatments which try to compensate for a deficiency in an endogenous gene (such as the bubble children example), which require permanent expression of a gene, or which require expression of a nucleic acid in a particular tissue type are relatively difficult. By contrast, treatments which require transiently expressing a protein in the blood are relatively simple. A protein can be delivered to the blood via a number of different cell types including muscle, liver cells, and fibroblasts. Also, the accumulation of a protein in the blood allows for easy assessment of its levels, and consequent

adjustment of dosage and/or frequency of administration to suitable levels. Indeed, for these reasons, it may be inaccurate to characterize the methods as gene therapy.

7. Although the approach described in the above application treats a condition of the brain, treatment can be effected using DNA immunization to deliver an antibody in the blood. Moreover, from my knowledge of a clinical trial involving active immunization with Aβ in which some beneficial effect was experienced from only a relatively short period of treatment notwithstanding considerable variation in antibody levels generated, it appears that even transient low level expression of antibody can be sufficient to have some benefit. I realize of course that the previous clinical trial actively immunized with Aβ protein, whereas the methods at issue here immunize with DNA encoding an antibody. Nevertheless, I believe a connection can reasonably be drawn between the two in predicting that relatively low level and transient expression of antibodies may exert some benefit as both have a goal of generating antibodies to Aβ in the blood. In my opinion, the use of DNA vaccination to deliver antibodies to the blood in such fashion represents a relatively undemanding form of gene therapy of a kind for which numerous clinical trials were approved in and around 1999. This form of gene therapy does not require experimentation to resolve issues, such as those relating to tissue specific expression or permanent expression, that have arisen in other forms of gene therapy.

8. As I have indicated by 1999, several delivery systems were well known and in use in clinical trials, including adenoviruses, adenoassociated viruses, retroviruses and naked DNA. I and other scientists in the field were well familiar with the types of vectors, regulatory systems and methods of delivery used in these approaches, and would not need a patent application to spell out these matters in great detail. Construction of vectors and testing in cell culture, and performing any necessary modifications to ensure a desired level of expression before commencing gene therapy were all considered routine practice in the field. In particular, deleting the E1 gene from adenoviral vectors was entirely common practice in the field before 1999, because of advantages of improved production (see, e.g., Alvarez et al., *Human Gene Therapy* 8(2), 229-42 (1997)). I also note that the constitutive CMV promoter disclosed in the application would be appropriate for expressing an antibody in any of a variety of tissues for ultimate delivery to the blood. Of the approaches described in the patent application, my

preference would have been to inject adenoassociated virus directly into the blood or to inject naked DNA into muscle. Both result in delivery of antibodies to the blood and neither is significantly affected by immunogenicity. Nevertheless, I believe other strategies would also work.

9. I understand the Examiner is of the view that difficulties with immunogenicity rendered gene therapy infeasible as of 1999. However, this was not the view of those in the field, such as me, at that date (or now), nor the FDA or similar regulatory bodies in other countries, nor as outlined above the federal government (i.e. the NIH sponsored very high profile Vaccine center which was directly praised this year by President Bush at theG8 summit) or the large pharmaceutical company Merck. Issues of immunogenicity were most relevant to the use of adenovirus. However, this did not stop adenovirus being used as the vector in numerous clinical trials. Immunogenicity was not a problem using retroviral vectors, adenoassociated virus or naked DNA.

10. I also understand that the Examiner doubts that antibodies could be successfully expressed following immunization with DNA and disregards the example provided by Arafat *et al.*, *Gene Therapy,* 9, 256-62 (2002) because it was published three years after the priority date of the present application. Arafat injects adenovirus encoding a single-chain antibody into the blood to express antibody. I see nothing of importance in the Arafat reference that was not known in the field of gene therapy by at least 1999. In particular, as I have noted, the practice of deleting E1 from adenoviral vectors was entirely standard in the field at that date. Moreover, others have successfully expressed injected DNA encoding an antibody previously (see Alvarez et al. Human Gene Therapy 8(2), 229-42 (1997)). Thus, I conclude that an antibody could have been successfully expressed by conventional gene therapy technology available in 1999.

11. In conclusion, I do not agree with the negative view of gene therapy in general portrayed by the examiner, nor with extent of problems alleged to exist in carrying out the methods of nucleic acids immunization described in the above patent application. The application describes a strategy of treating Alzheimer's disease using antibodies to Aβ and provides an example showing that this can be achieved by intravenous administration of a monoclonal antibody in a mouse

model. In my opinion, those in the field of gene therapy around 1999, including me, would have considered it plausible that a similar result could be achieved by using DNA immunization to deliver antibody to the blood, and that we could achieve this without excessive experimentation based on our existing knowledge of the field and the teaching of the specification.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

David Weiner, Ph.D.

Date 10/17/05

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 650-326-2400
Fax: 415-576-0300
Attachments
JOL:jol
60611290 v1

HUMAN GENE THERAPY 8:229–242 (January 20, 1997)
Mary Ann Liebert, Inc.

Clinical Protocol

A Phase I Study of Recombinant Adenovirus Vector-Mediated Delivery of an Anti-erbB-2 Single-Chain (sFv) Antibody Gene for Previously Treated Ovarian and Extraovarian Cancer Patients

STUDY CHAIRMEN: Ronald D. Alvarez, M.D.
Division of Gynecologic Oncology
Room 538, Old Hillman Building
618 South 20th Street
Birmingham, AL 35233-7333
PHONE: (205)-934-4986
FAX (205) 975-6174

David T. Curiel, M.D.
Gene Therapy Program
Room 620, Wallace Tumor Institute
1824 6th Avenue South
Birmingham, AL 35233-7333
PHONE: (205)-934-8627
FAX (205) 975-7476

1.0 Objectives

1.1 To determine the maximally tolerated single dose (MTD) of a recombinant adenovirus encoding an anti-erbB-2 single chain antibody gene (sFv) in previously treated ovarian and extraovarian cancer patients.

1.2 To determine the spectrum of toxicities encountered with and the safety of administration of a recombinant adenovirus encoding an anti-erbB-2 single chain antibody gene (sFv) in previously treated ovarian and extraovarian cancer patients.

1.3 To determine antitumor activity at the molecular level of a recombinant adenovirus encoding an anti-erbB-2 single chain antibody gene (sFv) in previously treated ovarian and extraovarian cancer patients.

2.0 Background and Rationale

2.1 Significance of Study

Epithelial ovarian carcinoma is the leading cause of death from gynecologic cancer in the United States. Approximately 24,000 new cases were estimated to occur in 1994 resulting in 13,600 deaths from this disease.[1] This figure exceeds the number of deaths from all other gynecologic malignancies combined. Primary papillary adenocarcinoma of the peritoneum (extraovarian carcinoma) is a distinct clinical entity that histologically and clinically mimics epithelial ovarian carcinoma.[2] Over 70% of these patients present with late stage disease, the majority of which cannot be completely resected at the time of initial surgery. Chemotherapy has become the primary adjunct to surgery in obtaining a complete clinical remission in ovarian cancer patients.

Many different classes of chemotherapeutic agents have demonstrated activity in ovarian cancer.[3] The demonstration that cisplatin was active in previously treated patients with recurrent ovarian cancer led to a series of prospective randomized clinical trials to determine the role of cisplatin in the primary treatment of ovarian cancer patients. The Gynecologic Oncology Group (GOG) performed the largest trial of a platinum-containing regimen versus a non-platinum regimen. Four hundred forty patients with suboptimally debulked Stage III and Stage IV ovarian carcinoma were randomized to receive doxorubicin plus cyclophosphamide, with or without cisplatin. The

clinical response rate of 51% in the cisplatin group was significantly higher than the 26% response rate in the group that did not receive cisplatin ($p < 0.0001$).[4] Several other studies have confirmed the increased response to platinum containing regimens and have lead to the standard use of platinum based regimens in patients with advanced ovarian cancer.[5, 6]

Paclitaxel is a new agent which has demonstrated considerable activity in patients with ovarian carcinoma. Based on the activity of paclitaxel as a second line agent, the GOG conducted a prospective randomized trial in advanced ovarian cancer comparing cisplatin and cyclophosphamide to cisplatin and paclitaxel. In an interim report of 290 evaluable patients, the clinical response rate was significantly higher for the cisplatin/paclitaxel arm (79%) than that achieved in the cisplatin/cyclophosamide group (63%).[7] This reported response advantage to the combination of cisplatin and paclitaxel, along with an increased supply of paclitaxel, has lead to the adoption of this regimen as first line therapy in many centers and as the standard arm in many ongoing Phase III clinical trials.

Although response to initial chemotherapy in ovarian cancer patients approaches 70%, most are transient and approximately 80% of patients (particularly those with advanced stage disease) will recur. A variety of salvage therapies have been evaluated. Paclitaxel appears to be the most effective second line agent for patients with persistent or recurrent ovarian cancer.[8] However, limitations in long term response and incorporation of paclitaxel into primary therapy has diminished the initial euphoria over paclitaxel as a salvage agent. Ifosphamide and hexamethylmelamine have demonstrated activity as salvage agents; however, response rates are approximately 20% and are rarely long term.[9, 10] Intraperitoneal consolidation chemotherapy has been investigated but the impact on long term survival is inconclusive.[11] Radiotherapy with P32 or whole abdominal radiation appears to have limited effectiveness, particularly in patients with large volume disease.[12]

Although a variety of salvage agents and strategies have been investigated, few have demonstrated long term effectiveness and the five-year survival of patients with stage III disease remains at best 15% to 30%. It is evident that advances in therapy are needed to improve survival in these patients. As an alternative, we have developed gene therapy strategies targeting ovarian carcinoma. The goal of this study is to evaluate one of these gene therapy strategies: recombinant adenovirus encoding the anti-erbB-2 sFv gene. Specifically, we will establish the MTD and evaluate the safety and toxicity of this method in previously treated ovarian and extraovarian cancer patients. In addition, we will determine if this gene therapy can accomplish any molecular evidence of antitumor activity in this patient population.

2.2 Background for Proposed Gene Therapy Strategy

The rationale for the present proposal is based upon several concepts, which are delineated herein:

2.21 Strategies to accomplish mutation compensation

The recognition of, and definition of, the molecular basis of carcinogenesis makes it rational to consider genetic approaches to therapy. In this regard, a number of strategies have been developed to accomplish cancer gene therapy [13–20]. One of these strategies is mutation compensation, in which gene therapy techniques are designed to rectify the molecular lesions etiologic of malignant transformation in the cancer cell.

One of the genetic lesions etiologic of malignant transformation is aberrant expression of "dominant" oncogenes. Aberrant expression of the corresponding oncogene product is thought to elicit the associated neoplastic transformation. Gene therapy strategies have been proposed to achieve genetic correction of this type of lesion. In this context, the molecular therapeutic intervention is designed to ablate expression of the dominant oncogene. The most universally employed methodology to achieve this end is the utilization of "anti-sense" molecules (DNA or RNA oligonucleotides) [21–24]. These molecules are designed to specifically target "sense" sequences to achieve blockade of the encoded genetic informational flow. A variety of experimental models have demonstrated the potential utility of the anti-sense approach as an anti-cancer therapeutic. Several studies have shown the ability to selectively ablate a dominant oncogene with reversion of the malignant phenotype.[25, 26] In selected instances, the in vivo demonstration of this effect could also be accomplished by direct, in vivo delivery of the anti-sense molecules.[27, 28] Thus, the antisense approach offers the potential to achieve targeted disruption of specific genes and potential anti-cancer effect.

Despite the potentially novel therapeutic strategies offered by the anti-sense approach, this methodology in practice is associated with severe limitations which has limited wide employment of this technology in human gene therapy anti-cancer protocols. In this regard, it has been difficult to block expression of a great many cancer-related genes with anti-sense techniques. In addition, delivery of the anti-sense molecules has been highly problematic.[21–23] The tumor environment is deleterious to these unstable molecules and it is often difficult to achieve effective intracellular levels. To circumvent this problem, a number of design modifications of the anti-sense molecules have been developed to enhance their stability.[21–23, 29] In addition, a number of vector approaches have been explored for effective cellular delivery.[27, 30, 31] Despite these various maneuvers, the over-riding limitations to the employment of this promising therapeutic modality remains the idiosyncratic efficacy of specific anti-sense for a given target gene and the suboptimal delivery of anti-sense molecules.

2.22 Intracellular antibodies to achieve selective erbB-2 ablation

As an alternative method to achieve targeted gene "knock-out" we have developed a highly novel methodology. In this regard, we have been able to construct a gene encoding a single-chain immunoglobin (sFv) directed against a specific oncogene. The resultant recombinant molecule, when expressed in prokaryotic systems, could yield a single-chain antibody (sFv) which retained the antigen recognition and binding profile of the parent immunoglobin. We wondered whether it might also be possible to exploit the exquisite specificity of immunoglobins for selected gene knock-out in a distinct manner. We chose to target the erbB-2 oncogene. ErbB-2 is a 185-kDa transmembrane protein kinase receptor with extensive homology to the family of epithelial growth factor receptors.[32] Several lines of evidence suggest that aberrant expression of the erbB-

2 gene may play an important role in neoplastic transformation and progression. In this regard, ectopic expression of erbB-2 has been shown to be capable of transforming rodent fibroblasts in vitro.[20] In addition, transgenic mice carrying either normal or mutant erbB-2 develop a variety of tumors, predominantly neoplasms of mammary origin.[19] Importantly, it has been shown that amplification and/or over-expression of the erbB-2 gene occurs in a variety of human epithelial carcinomas, including malignancies of the breast, ovary, gastrointestinal tract, salivary gland, and lung.[16–18] In the instances of breast and ovarian carcinoma, a direct correlation has been noted between over-expression of erbB-2 and aggressive tumor growth with reduced overall patient survival.[15, 24] A method was thus conceptualized whereby intracellular expression of the anti-erbB-2 sFv could inactivate the erbB-2 gene product. In this regard, it was hypothesized that if an anti-erbB-2 sFv were localized to the endoplasmic reticulum (ER) of the target SKOV3 cells, the nascent, newly synthesized erbB-2 protein would be entrapped within the ER of the cells, and unable to achieve its normal cell surface localization. It was further hypothesized that this intracellular entrapment would prevent the erbB-2, a transmembrane tyrosine kinase receptor, from achieving interaction with its ligand, thus abrogating the autocrine growth factor loop driving malignant transformation in erbB-2 over-expressing cell lines.

2.23 The preliminary data which relates to these studies

2.231 Intracellular expression of anti-erbB-2 single chain immunoglobulin (sFv) results in down-regulation of cell surface erbB-2 expression in SKOV3 cells

As a means to prevent maturational processing of the nascent erbB-2 protein during synthesis, a gene construct was designed which encodes an anti-erbB-2 single chain immunoglobulin (pGT21) (Figure 1 [Ed. Note: Figures not reproduced except for Fig. 19.]). It was hypothesized that expression of this construct in target cells would result in an ER localized form of the sFv which would entrap erbB-2 during synthesis thus preventing its subsequent translocation to the cell surface. As a control, a similar anti-erbB-2 sFv was designed, which lacked a signal sequence which would dictate its localization to the ER (pGT20). These sFv constructs were cloned into the eukaryotic expression vector pCDNA3, which directs high level heterologous gene expression from the CMV early intermediate promoter/enhancer. For this analysis, the plasmid DNAs pCDNA3, pGT20, and pGT21 were transfected into the erbB-2 over-expressing human ovarian carcinoma cell line SKOV3 using the adenovirus-polylysine (AdpL) method (Figure 2). Preliminary experiments demonstrated that the adenovirus-polylysine-DNA complexes containing a β-galactosidase reporter gene (pCMVβ) accomplished detectable levels of reporter gene expression in >95% of targeted cells. At various times after transfection, the cells were evaluated for cell surface expression of erbB-2 using the technique of immunohistochemistry employing an anti-human erbB-2 monoclonal antibody. Cells transfected with the irrelevant plasmid DNA pCDNA3 exhibited high levels of cell surface erbB-2, as would be expected (Figure 3). Additionally, SKOV3 cells transfected with the non-ER form of the anti-erbB-2 sFv(pGT20) exhibited levels of cell surface erbB-2 similar to the pCDNA3 control. In marked contrast, SKOV3 cells transfected with pGT21, which encodes an ER form of the anti-erbB-2 sFv, demonstrated marked down-regulation of cell surface erbB-2 expression. This down-regulation appeared to be time-dependent with cell surface erbB-2 levels progressively declining from 48 to 96 hours post-transfection. At 96 hours post-transfection, fewer than 10% of the pGT21 transfected cells exhibited detectable levels of cell surface erbB-2 protein. The cells otherwise appeared morphologically indistinguishable from the control groups.

2.232 Intracellular expression of anti-erbB-2 single-chain immunoglobulin (sFv) results in marked inhibition of cellular proliferation in SKOV3 cells

To determine whether cell surface expression of erbB-2 was correlated with cellular proliferation rates, the effect of the various gene constructs on tumor cell proliferation was evaluated. For this analysis, immunohistochemistry for the proliferation associated antigen Ki-67 was employed (Figure 4). Transfection of cells with the control plasmid pCDNA3 resulted in the immunohistochemical detection of active cellular proliferation. In addition, transfection with the non-ER form of the anti-erbB-2 sFv (pGT20) did not result in any net change in cell proliferation. In marked contrast, transfection of the erbB-2 over-expressing cell line SKOV3 with the ER form of the anti-erbB2 sFv resulted in a dramatic inhibition of cellular proliferation.

The degree of inhibition of cell proliferation was also assessed employing a quantitative assay (Figure 5). Control studies had established a linear relationship between the number of proliferative cells and the concentration of formazan released. Transfection of SKOV3 cells with the pCDNA3 plasmid did not affect the measured index of cellular proliferation when compared to non-transfected cells ($p = 0.103$). Additionally, cells transfected with the non-ER form of the anti-erbB-2 sFv did not significantly differ from these two controls ($p = 0.118$). However, transfection of SKOV3 with the ER form of the anti-erbB-2 sFv resulted in a very significant inhibition of cellular proliferation ($p < 0.001$). Extrapolation of the measured absorbance against the standard curve indicated that cellular proliferation was inhibited more than 95% compared to the control groups. Thus, the expression of the ER form of the anti-erbB-2 sFv inhibits proliferation of these erbB-2 over-expressing tumor cells. It is of interest that the level of down-regulation of cell surface erbB-2 mediated by the ER form of the anti-erbB-2 sFv is paralleled by the magnitude of the observed anti-proliferative effects.

2.233 Intracellular expression of anti-erbB-2 single-chain immunoglobulin results in marked reduction in survival of neoplastic cell clones

As the ER anti-erbB-2 sFv exhibited such a prominent anti-proliferative effect, it was hypothesized that it might also exhibit a direct tumoricidal effect in cells modified to stably express this gene construct. As the plasmids pCDNA3, pGT20 and pGT21 contained neomycin selectable markers, they were used to derive stable clones. As a preliminary control, the various plasmid con-

structs were utilized to derive G418 resistant clones in HeLa cells, a cancer cell line not characterized by over-expression of erbB-2. After selection, the number of clones derived from transfection with pGT20 and pGT21 was not significantly different from the cells transfected with the control plasmid pCDNA3.(Table 1) A similar analysis as then carried out with the erbB-2 over-expressing tumor line SKOV3 as the target. In this study, the number of clones derived with pGT20 did not differ from the number derived with the control plasmid pCDNA3.(Table 1) Transfection with pGT21, however, resulted in a dramatic reduction in the number of stable clones derived ($p < 0.001$). It thus appeared that the expression of the ER form of the anti-erbB-2 sFv was incompatible with long term viability of transfected SKOV3 cells. Further, this effect appeared to be specific for erbB-2 over-expressing cells as this differential survival was not noted for HeLa cells. A similar analysis was carried out on another tumor target, the ovarian carcinoma cell-line SW626. This cell line is also known to over-express cell surface erbB-2, however, not at the same magnitude as for the SKOV3 cells. In this study, the ER anti-erbB-2 sFv also showed a significant reduction in the number of stable clones when compared to the non-ER form of the anti-erbB-2 sFv ($p = 0.020$). The magnitude of this effect, however, was substantially less than that observed for SKOV3. It thus appears that the level of anti-neoplastic effect achieved by the anti-erbB-2 sFv is correlated with the level of cell surface erbB-2 over-expression on target cells.

2.234 *Intracellular expression of anti-erbB-2 single-chain immunoglobulin (sFv) results in abrogation of tumorogenicity of SKOV3 cells in soft agar and in xenografted nude mice*

Anchorage independent growth in soft agar is considered a marker of malignant transformation and tumorigenicity. To test the effect of anti-erbB-2 sFvs on anchorage independent growth, we assayed SKOV3 cells for their ability to grow on soft agar after transient transduction with the plasmid DNA constructs carrying the anti-erbB-2 sFv gene. SKOV3 cells transduced with control DNA pCDNA3 and cytosolic anti-erbB-2 sFv, pGT20, exhibited more than 20 fold higher colony-forming efficiency than the group transduced with the ER directed anti-erbB-2 sFv, pGT21.(Figure 6) In this regard, the ER form of the anti-erbB-2 sFv was found to exert a marked anti-neoplastic effect on SKOV3 cells resulting in an arrest of anchorage independent growth on soft agar. Thus, reversion of malignant phenotype was achieved *in vitro* in the human ovarian carcinoma cell line SKOV3.

Formation of xenogeneic tumors in the soft tissues of immunodeficient mice is an additional index of tumorigenicity. Thus, an additional experimental test was performed to assess the ability of transfected ovarian cancer cells to form tumors in nude mice. Human ovarian cancer cells, SKOV3, were transiently transduced with either the relevant, ER form of the anti-erbB-2 sFv or the control constructs, and subsequently injected subcutaneously (s.c.) into nude mice to determine their tumorigenic potential. A significant time-dependent increase in tumor volumes was noted in animals injected with tumor cells transduced with the control construct and the cytosolic anti-erbB-2 sFv.(Figure 7) In marked contrast, complete tumor eradication was achieved 80 days after s.c. transplant in the group challenged with the ER directed anti-erbB-2 sFv. Thus reversion of malignant phenotype was achieved *in vivo* in xenogeneic tumor grafts as indicated by this additional assay of tumorigenicity.

2.235 *Intracellular expression of anti-erbB-2 single-chain immunoglobulin (sFv) results in a cytocidal effect by inducing apoptosis*

Plasmid DNAs which encoded either the cytosolic form of the ER-form of the anti-erbB-2 sFv were delivered to SKOV3 cells, as well as the control plasmid pCDNA3. Transfected cells were evaluated for growth kinetics by analysis of the time-dependent increase in cell number. It could be noted in this analysis that cells transfected with the irrelevant plasmid DNA, pCDNA3, as well as the cytosolic pGT20 showed a time-dependent increase in cell number.(Figure 8) In marked contrast, transfection with the ER-form of the anti-erbB-2 sFv, pGT21, resulted in a significant inhibition of cell growth. Analysis of the cell growth in this group demonstrated a kinetic pattern consistent with tumor cell eradication rather than with growth arrest. Thus, analysis of cell growth kinetics suggested that intracellular expression of the ER-form of the anti-erbB-2 sFv was cytocidal to erbB-2 over-expressing tumor cells, and not cytostatic as initially postulated.

To more accurately determine the precise biologic effect of anti-erbB-2 sFv expression in the erbB-2 overexpressing tumor target, direct analysis of cell viability was studied. For this analysis, the XTT assay was employed. This method provides a direct determination of cell viability by quantifying a specific cellular enzymatic reaction.[33] For this analysis, the ovarian carcinoma line SKOV3 was transfected as before with the plasmid DNAs pCDNA3, pGT20 and pGT21. Transfection with pGT21 resulted in a time-dependent decrease in cell viability. This effect was demonstrated to result in a >95% decrement in the number of viable cells in this group by 72 hours post-transfection.(Figure 9) Transfection with the control plasmids pCDNA3 and pGT20, however, did not exert any significant effect on cell viability. These results therefore confirm our previous study demonstrating that the ER form of the sFv uniquely elicits phenotypic alterations in the SKOV3 cells.

The foregoing studies are consistent with the concept that entrapment of erbB-2 within the ER of erbB-2 over-expressing tumor cells triggers their eradication. However, this effect may not simply be based on erbB-2 down-regulation, as anti-sense inhibition of erbB-2 gene expression can elicit proliferative arrest of erbB-2 over-expressing cells, but not their killing.[34, 35] The high level of specificity of this effect in erbB-2 over-expressing cells suggested that the basis of this may be the triggering of a program for induced cell death in these tumor targets. Thus, to further delineate the mechanistic basis of this effect, studies were carried out to determine if programmed cell death, or apoptosis, was occurring. As before, the plasmid DNA constructs pCDNA3, pGT20 and pGT21 were delivered to the erbB-2 over-expressing SKOV3 cells and the non-erbB-2 expressing tumor cell line HeLa. At specific time points post-transfection, cells were harvested and evaluated for evidence of nuclear DNA fragmentation, a hallmark of programmed cell death.[36, 37] In the HeLa cells, transfection with the various constructs did not result in any evidence of apoptotic cellular events as determined by morphologic appearance or alterations in DNA as measured by gel electrophoresis.(Figure 10A) Transfection of the SKOV3 cells

with the control plasmid pCDNA3 and the cytosolic anti-erbB-2 sFv pGT20 similarity did not elicit any evidence of cellular apoptosis. When the SKOV3 cells were transfected with the ER form of the anti-erbB-2 sFv, however, marked changes in chromosomal DNA were noted. These changes were first detected at 48 hours post-transfection and revealed on 2% agarose gel as a characteristic apoptotic ladder with fragmentation of DNA into segments differing in molecular weight by the approximate magnitude of 200 bp.(Figure 10B)

As an additional assay of apoptosis, the presence of apoptotic nuclei was evaluated employing differential nuclear uptake of DNA-binding dyes.[38] In this analysis, it could be seen that SKOV3 cells transfected with the plasmid DNA pGT21 showed intense nuclear staining characteristic of cellular apoptosis. These alterations were not seen in cells transfected with the control plasmids pCDNA3 and pGT20.(Figure 11A–C) Quantitative analysis demonstrated that >90% of the transfected SKOV3 cells exhibited apoptotic nuclear changes, whereas cells transfected with pCDNA3 and pGT20 did not exhibit a level of apoptosis different from untransfected controls.(Figure 11D) Thus, the basis of the cytocidal effect of the ER anti-erbB-2 sFv in the erbB-2 over-expressing cells was the induction of cellular apoptosis.

2.236 *Intracellular expression of anti-erbB-2 single-chain immunoglobulin (sFv) results in marked reduction in survival of primary ovarian cancer cells*

As previously demonstrated, intracellular expression of the ER form anti-erbB-2 sFv was cytocidal to erbB-2 overexpressing tumor cells, and not cytostatic as we had initially postulated.[39] The next step in establishing the potential utility of this approach in a clinical context was to treat primary human tumor material. To establish the biologic effect of the anti-erbB-2 sFv in primary cultures, erbB-2 expressing ovarian carcinoma cells (OVCA2) were isolated and cultured from human malignant ascites. The XTT assay was employed for direct determination of cell viability with anti-erbB-2 sFvs. The human ovarian carcinoma cell line, SKOV3, was used as a positive control. OVCA2 and SKOV3 cells were transfected as before with plasmid DNAs. The ER directed anti-erbB-2 sFv elicited a significant cytotoxic effect in both of these cells 96 hrs post-transfection. (Figure 12). The magnitude of the cytocidal effect observed in primary cultures was comparable to that observed in the cell line known to overexpress erbB-2. Control experiments clearly demonstrated that this effect was highly specific for erbB-2 overexpressing cell lines and was not observed in cells transduced with the cytosolic sFv or pCDNA3 construct. Thus, inhibition of erbB-2 in primary cultures by intracellular sFv resulted in complete tumor cell eradication. Importantly, this result demonstrated the utility of this method of targeted tumor eradication in the context of human disease.

2.237 *Transfection of anti-erbB-2 single-chain immunoglobulin (sFv) is more efficiently achieved with a recombinant adenovirus vector in vivo*

To accomplish gene therapy for ovarian carcinoma by this approach, gene delivery must be achieved to tumor cells in situ by direct, in vivo application. To determine the optimal vector for this strategy, direct *in vivo* gene delivery was studied in a human tumor xenograft model. For this analysis athymic nude mice were challenged intraperitoneally with 1×10^7 SKOV3 cells to establish an orthotopic model of human ovarian carcinoma. After 48 hours, vectors containing an E. Coli galactosidase (lacZ) reporter construct were administered intraperitoneally in the study animals. Evaluated vector systems included adeno-polylysine complexes (AdpL), recombinant adenovirus (AdCMVlacZ), and cationic liposomes (DOTAP). After an additional 48 hrs, the peritoneal contents were harvested and human ovarian carcinoma cells were evaluated for the expression of the lacZ reporter gene. In this analysis, the highest level of in situ transduction was accomplished by the recombinant adenovirus.(Figure 13) Thus, a recombinant adenovirus will be employed for direct in vivo delivery of the anti-erbB-2 sFv gene to the peritoneum.

2.238 *Construction and in vitro analysis of a recombinant adenovirus encoding the anti-erbB-2 sFv*

As the optimum vector for in situ transduction of ovarian carcinoma cells was shown to be the adenovirus, a replication-defective, recombinant adenovirus was constructed encoding the anti-erbB-2 sFv.(Figure 14) To establish that the anti-erbB-2 sFv gene functioned in this vector configuration, analysis of the recombinant anti-erbB-2 encoding adenovirus was carried out. For these studies, the adenovirus encoding the ER-form of the anti-erbB-2 sFv (Ad21) was delivered to the erbB-2 over-expressing human ovarian carcinoma cell line SKOV3. Cells were analyzed for viability employing the XTT assay as previously described. In this analysis, it could be seen that the anti-erbB-2 sFv encoding adenovirus exerted the same cytocidal effect in the erbB-2 over-expressing targets as observed with the AdpL-mediated delivery.(Figure 15) Thus, a replication-defective adenovirus encoding the anti-erbB-2 sFv has been constructed for the application of direct, in situ gene transfer to ovarian carcinoma cells.

2.239 *Efficacy of recombinant adenovirus encoding anti-erbB-2 sFv in an in vivo orthotopic murine model*

To establish the utility of the adenoviral vector for this application, we constructed an orthotopic murine model employing human tumor xenografts with SKOV3 cells. Forty-eight hours after engraftment with SKOV3 cells, SCID mice were challenged intraperitoneally with the E1A-deleted recombinant adenovirus encoding the anti-erbB-2 sFv (Ad21) or an E1A-deleted recombinant adenovirus encoding the irrelevant reporter gene lacZ (AdCMVlacZ). Ninety-six hours after treatment, the animals underwent peritoneal lavage for analysis of harvested tumor cells. It could be seen in this analysis that the number of viable cells was dramatically decreased in the Ad21 group compared to the AdCMVlacZ group.(Figure 16A) Analysis of the mechanism of cell death demonstrated that the Ad21 virus induced cellular apoptosis in vivo, as had been demonstrated in vitro.(Figure 16B) Thus the recombinant adenovirus encoding the anti-erbB-2 sFv accomplished a specific cytocidal effect in an orthotopic murine model of human ovarian cancer.

2.24 Highly selective gene knock-out employing intracellular single-chain antibodies

These studies have allowed the definition of a potential therapeutic modality for the achievement of selective eradication of genetically aberrant target cells. In the context of anti-cancer therapeutics, it has been proposed that selective abrogation of specific target genes can revert neoplastic cells from the malignant phenotype. In this regard, the over-expression of dominant oncogenes has been shown to be a critical determinant of neoplastic transformation and progression. Targeted disruption of these genes, in selected instances, may accomplish reversion from this malignant phenotype or initiation of cell death. This has been accomplished utilizing strategies of anti-sense and transdominant mutations in both in vitro and in vivo experimental models. The utilization of intracellular single-chain immunoglobulins represents a novel strategy for this purpose. This methodology offers significant potential advantages over previous genetic techniques for therapeutic gene ablation. In this regard, many monoclonal antibodies (mAbs) have been developed against a variety of cancer related gene products. It would thus be logical to convert these reagents to sFvs which would thus possess the potential for therapeutic utility. As in the example reported here, expression of intracellular immunoglobulins may possess the potential to achieve a highly selective effect on specific gene products with the end result of targeted tumor eradication. This high level of specificity would predict that these agents would be selectively toxic for cancer cells expressing the relevant target oncogene. This high level of specificity would therefore allow the achievement of a high therapeutic index for cancer therapeutics.

2.241 Safety of the approach using an anti-erbB-2 sFv encoding recombinant adenovirus in the context of ovarian cancer gene therapy

One of the theoretical advantages of this approach is the targeted nature of this intervention. There are two potential safety issues relating to this protocol. The first issue relates to ectopic localization of the intraperitoneally delivered adenovirus. In this regard, previous studies by Crystal et al[40] and Abelda et al[41] have demonstrated that recombinant adenoviral vectors delivered by this route in murine models remain localized to the peritoneal cavity. In Crystal's study, sensitive PCR techniques could not demonstrate any adenovirus beyond the peritoneal cavity.[40] In Abelda's study, it was confirmed that intraperitoneally delivered adenovirus was confined to the peritoneum.[41] The small amount of ectopic expression noted was within the surrounding mesothelium and within hepatocytes. Therefore, to determine the potential deleterious effects of anti-erbB-2 sFv expression at these sites, transduction of human hepatoma and mesothelioma cell lines was accomplished. In these studies, it could be demonstrated that expression of the anti-erbB-2 sFv had no cytocidal effects in these cellular targets.(Figure 17) Therefore, we do not expect any significant level of ectopic localization of the intraperitoneally delivered adenovirus on the basis of the extensive studies of Crystal and Abelda.[40, 41] In the context whereby some ectopic transduction might occur, we have shown that expression of the anti-erbB-2 sFv will not be deleterious to these cellular targets.

In addition, direct toxicity of the anti-erbB-2 sFv encoding recombinant adenovirus was evaluated. For these studies, SCID mice were challenged intraperitoneally with various doses of the Ad21 vector. Seven days post challenge, animals were studied for organ level pathology by gross and microscopic analysis. In these studies, no animal suffered untoward clinical effects related to the viral vector. In addition, no organ level pathology could be attributed to the anti-erbB-2 sFv encoding recombinant adenovirus.

2.3 Application in ovarian cancer

ErbB-2 has been established to be a transmembrane protein tyrosine kinase receptor.[42] Candidate ligands specific for this receptor have been identified; however, the precise mechanisms of receptor-ligand interaction remain to be established. Cell surface over-expression of the erbB-2 protein has been linked to aberrant cellular proliferation.[43–45] The basis for this phenomenon has been putatively based upon the ability of erbB-2 to accomplish tyrosine kinase-mediated signal transduction after interaction with its cognate ligand.

Overexpression of erbB-2 has been shown to be important in the pathogenesis of a large subset of ovarian carcinomas.[43–45] In addition, in these instances, the overexpression of erbB-2 has been shown to be correlated with a more aggressive tumor with worse overall prognosis.[46, 47] Thus, ovarian carcinoma is an ideal target for initial studies designed to evaluate the therapeutic efficacy of gene delivery strategies employing intracellular single-chain-mediated erbB-2 knock-out.

The prominent down-regulation of cell-surface erbB-2 would be predicted to significantly attenuate its ability to interact with its cognate ligand. This effect would be further anticipated to impair ligand-induced receptor transduction with a negative effect on cellular proliferation. The correlation between cell surface erbB-2 expression and cellular proliferation, as noted in the previously described preliminary data, is consistent with the concept that receptor-ligand interaction is crucial to the ability of erbB-2 to promote cellular proliferation.

It is highly appropriate to investigate this novel therapy in recurrent ovarian and extraovarian cancer patients. This population of patients has no available curable therapeutic options. The proposed gene therapy strategy employing an anti-erbB-2 sFv gene has been highly promising in animal studies. All gene therapy trials in humans thus far have been Phase I studies. Therefore, the intent of this proposal is to primarily establish the toxicity and safety and secondarily determine molecular efficacy of this promising therapy. Completion of this protocol will allow development of Phase II trials which will identify clinical efficacy.

3.0 Study Modalities and Safety Information

3.1 Formulation of anti-erbB-2 sFv gene construct

An expression plasmid was derived containing a gene construct encoding a single chain immunoglobin directed against human erbB-2. For this purpose, the eukaryotic expression vector pCDNA3 (Invitrogen) was used which drives transcription via the CMV early intermediate promoter/enhancer and contains a neomycin resistance gene as a selective marker. The anti-erbB-2 sFv plasmid e23scFv was obtained from Oncologix and used for the derivation of subsequent constructs. Genetic modifica-

tions of the anti-erbB-2 sFvs were carried out employing polymerase-chain reaction (PCR) methods. PCR was performed using Pfu polymerase (Strategene) with the 5′ primer: AGGGTACCATGGACGTCCAGCTGACC, and the 3′ primer: GCTCTAGATTAGGAGACGGTGACCGTGGTCC. The PCR product, containing an ATG initiation codon followed by the sFv gene, was subject to digestion with the restriction endonucleases KpnI and XbaI, and cloned into pCDNA3. To ensure that the sFv was directed to the ER, the coding sequence for a leader peptide (MKSHSQVFVFLLLCVSGAHG) was incorporated into the 5′ end of the anti-erbB-2 sFv coding sequence by PCR methods. This PCR product was cloned into the KpnI/XbaI sites of pCDNA3. This construct is named pGT21. A replicative-defective adenovirus encoding the ER form of the anti-erbB-2 sFv gene was constructed as shown in Figure 14.

3.2 Preparation

Recombinant adenoviral vectors have been developed which are replication-defective based upon genomic deletions in the E1 region. These have been employed in a variety of approved and ongoing clinical trials. The adenoviral vector employed for this study is constructed in a matter consistent with these previous studies. The propagation of the recombinant adenoviral vector is carried out on the transcomplementing cell line 293. This is accomplished as shown in Figure 19.

3.3 Storage

Prepared adenoviral vector will be diluted to a concentration of 1×10^{11} plaque forming units/ml and stored in aliquots at $-70°C$ until use. The storage will be in 500 μl aliquots in sterile, individual capped vials.

3.4 Supplier

We have previous experience in preparing human use vector reagents for clinical gene therapy protocols. We will seek a FDA IND for local production of our adenoviral vector and all work will be carried out within FDA guidelines.

3.5 Administration

Recombinant adenovirus encoding the anti-erbB-2 sFv gene will be administered intraperitoneally through a percutaneous Tenckhoff catheter. The catheter will be placed as a secondary procedure at the time of laparotomy or as a primary procedure at a subsequent operation. The Tenckhoff catheter will be inserted into the peritoneal cavity, tunnelled through the subcutaneous tissue, and exteriorized through a percutaneous stab incision in the lower abdominal wall.

On the day of therapy, 1000 ml of saline will be infused with 5 mCi of Tc-aggregated albumin with nuclear imaging to assure adequate distribution. The patient will then receive an intraperitoneal bolus infusion of the assigned dose of anti-erbB-2 adenovirus complex followed by infusate of an additional 500 ml of saline.

3.6 Peritoneal Aspiration and Tumor Biopsy

At various specified points in the study, peritoneal contents will be harvested for evaluation. A tumor biopsy will be done at the time of Tenckhoff catheter placement. Peritoneal aspiration will be accomplished by infusion of 1000 ml of saline at the time of placement or through the Tenckhoff catheter followed by immediate withdrawal employing manual suction. Previous studies of intraperitoneal chemotherapy have demonstrated the feasability of retrieval of carcinoma cells for cytologic evaluation.[48, 49] Harvested cellular material will be used for studies of safety and molecular efficacy of the gene therapy protocol.

4.0 Patient Eligibility and Exclusions

4.1 Eligibility Criteria

4.11 Patients must have a histologically documented epithelial ovarian carcinoma or extraovarian adenocarcinoma.

4.12 Patients must have persistent or recurrent disease after standard debulking/staging surgery and first or second-line chemotherapy.

4.13 Patients must have immunohistochemical or ELISA evidence of erb-B2 over-expression in the most recently available tumor specimen.

4.14 Patients must be 18 years or older.

4.15 Patients may have measurable or nonmeasurable macroscopic residual disease. Disease must be confined to the peritoneal cavity ± retroperitoneal nodes.

4.16 Patients must have a GOG performance status of 0, 1, or 2, and have a life expectancy of greater than 3 months.

4.17 Patients must have adequate hematologic, renal, cardiac, and hepatic function defined as:

WBC > 3,000 μl
Granulocytes > 1,500 μl
Platelets > 100,000
Creatinine < 2.0 or Creatinine clearance > 60 mg/dl
Serum transaminases < 3× upper limits of normal
Normal serum bilirubin

4.18 Patients must have signed informed consent.

4.2 Exclusion Criteria

4.21 Patients with epithelial tumors of low malignant potential, stromal tumors, and germ cell tumors of the ovary are ineligible to participate in the study.

4.22 Patients who have been diagnosed with a prior malignancy with the exception of basal cell carcinoma of the skin are ineligible to participate in the study.

4.23 Patients who have received more than two prior chemotherapy regimens or prior irradiation are ineligible to participate in the study.

4.24 Patients with nonmeasurable microscopic residual disease are ineligible to participate in the study. Patients with intraabdominal disease >5 cms in diameter, intrahepatic disease or disease beyond the abdominal cavity are ineligible to participate in the study.

4.25 Patients who are pregnant or lactating are ineligible to participate in the study.

4.26 Patients with a GOG performance status of 3 or 4 are ineligible to participate in the study.

4.27 All potential candidates for the study will be tested for HIV infection. Those who are HIV positive are ineligible to participate in the study.

4.28 All potential candidates will be assessed for adequacy of intraperitoneal fluid distribution. Those potential candidates with <75% distribution are ineligible to participate in the study.

5.0 Treatment Plan

5.1 The study will identify the maximal tolerated single dose of recombinant adenovirus encoding the anti-erbB-2 sFv gene. After completing pretreatment evaluation (Section 6.1) and fulfilling the eligibility criteria (Section 4.0), patients will be treated with the assigned single dose of recombinant adenovirus as described in Section 3.5. All patients will be treated in the General Clinical Research Center (GCRC).

The dose range of recombinant adenovirus is based upon analogy to similar studies in humans evaluating Phase I parameters related to recombinant adenovirus vectors. The dose range of recombinant adenovirus encoding the anti-erbB-2 sFv gene in this study contains amounts of replication incompetent adenovirus resulting in the net delivery of a range of adenovirus particles paralleling those in previously approved human gene therapy protocols. In these previous studies, more than 20 patients at five centers have received recombinant adenoviral vectors.

5.2 Doses will be escalated in successive cohorts of patients according to the following schedule:

Group	*Plaque forming units*
1	10^8
2	10^{10}
3	10^{12}

5.3 Parameters for Dose Escalation

5.31 Temporal delay between treatment of new patients

The last patient entered at a dose level must be observed for at least 3 weeks after therapy prior to enrolling the first patient at the next dose level.

5.32 Number of patients per dose level

At least three patients will be treated at each dose level at which no dose-limiting toxicities occur. If dose-limiting toxicity occurs in one of the first 3 patients at a dose level, then additional patients (six if MTD not clearly exceeded before six are treated) will be treated at that dose level. If 0/3 or 1/6 patients at a dose level experience dose-limiting toxicity then dose escalation may proceed to the next level.

If 2 patients at a dose level have experienced dose-limiting toxicity, then the study will be closed.

5.4 There will be no intrapatient dose escalation.

6.0 Treatment Evaluation

6.1 Pretreatment Evaluation

The following observations and tests shall be performed within the week prior to initiating treatment: history and physical, toxicity grading, assignment of performance status, laboratory (CBC with differential, platelet count, chemistry profile, CA-125, anti-adenovirus type 5 antibody titer, and HIV titer), CT of abdomen and pelvis, and tumor measurements (in two dimensions, if measurable). A peritoneal aspirate and tumor biopsy will be obtained to provide baseline data for studies of molecular efficacy and safety.

6.2 Evaluation during Treatment

6.21 Evaluation for toxicity

Patients will be observed for 24 hours in the GCRC after administration of recombinant adenovirus encoding the anti-erbB-2 sFv gene. Any acute toxicity will be recorded. The following observations and tests shall be performed weekly for eight weeks after treatment: history and physical, toxicity grading, assignment of performance status, laboratory (CBC with differential, platelet count, chemistry profile including renal and liver function tests, CA-125, and anti-adenovirus type 5 antibody titer).

6.22 Evaluation for safety

In addition to clinical toxicities, vector-associated, cellular toxicities will be evaluated in this study. The employment of a replication-defective adenovirus in this study raises two relevant vector-related safety issues: 1) replication of the genomic-deleted adenovirus due to trans-complementing cellular factor and 2) recombination of the genomic-deleted adenovirus with generation of wild-type adenoviral particles. Cellular material harvested from the peritoneum at time points specified in Section 6.25 will be briefly established in primary culture. Cellular supernatants will then be collected and evaluated for context of adenovirus. Titering on the E1 trans-complementing cell line 293 will be employed to evaluate the replication of the E1-deleted virion in tumor material. Titering on the cell line HeLa will be employed to evaluate the derivation of wild-type adenoviruses through recombinantional events.

6.23 Evaluation of molecular response

Efficacy of the gene transfer and gene therapy treatment will be evaluated at the molecular level employing tumor material derived from the patients. In this regard, tumor cells harvested from the peritoneum at time points specified in Section 6.25 will initially be analyzed for efficacy of anti-erbB-2 sFv gene transfer to ovarian cancer cells in situ. This analysis will allow confirmation of the biologic consequences of gene transfer at the cellular level as well as provide an index of transduction frequency. These studies will confirm the efficacy of anti-erbB-2 sFv gene transfer by molecular biology and immunocytochemistry analysis of tumor cells for: 1) the presence of the anti-erbB-2 sFv DNA, 2) the presence of mRNA transcripts corresponding to the encoded sFv gene, and 3) anti-erbB-2 sFv protein corresponding to the encoded sFv gene.

Tumor cells will subsequently be evaluated for the predicted effects of the sFv gene transfer by employing the assays described in the preliminary data section. Specifically, tumor cells will then be evaluated for: 1) cell surface erbB-2 expression by immunocytochemistry and ELISA, and 2) cellular proliferation by Ki-67 immunocytochemistry and MTT assay. These studies will allow evaluation of the effects of sFv erbB-2 knock-out in the context of an intact patient in a human gene therapy protocol. They will provide preliminary evidence of the potential efficacy of this therapy.

6.24 Evaluation of clinical efficacy

At the eighth week, tumor measurements (in two dimensions), a CT of abdomen and pelvis will be performed.

6.25 Summary of Observations and Tests

The following observations and tests are to be performed and recorded before, during, and after treatment for patients enrolled in Part A:

Parameter	*Pre-Rx*	*Day 1*	*Days 4, 7, 14, 21, 28, 35, 42, 49*	*Day 56*
History/Physical	X	X	X	X
Weight/Performance Status	X	X	X	X
HIV	X			
Toxicity Grading	X	X	X	X
CBC/diff/Plt	X	X	X	X
Chem Profile[1]	X	X	X	X
Peritoneal aspirate	X		X	X
Antiviral AB Titers	X		X	X
Ca-125	X		X	X
Tumor Measurements	X			X
CT Abdomen/pelvis	X			X

[1]Chemistry profile to include Electrolytes, BUN, Creatinine, SGOT, Bilirubin, Alkaline Phosatase.

7.0 Study Parameters

7.1 Toxicity Grading

Gynecologic Oncology Group Toxicity criteria (Appendix I) will be used for grading of toxicity. Adverse reactions not included in these Criteria will be graded as follows:

Description	*Grade*
Mild	1
Moderate	2
Severe	3
Life-threatening	4
Lethal	5

7.2 Definition of Dose Limiting Toxicity (DLT)

For the purpose of this study, DLT will be defined as any grade 3 or greater non-hematologic toxicity other than nausea, vomiting, or fatigue. Hematologic toxicity will be considered dose-limiting if it produces a platelet count <20,000, an absolute neutrophil count (ANC) <500 for >5 days, if the patient is admitted to the hospital for a neutropenic fever, or if there is delay of the initiation of the second course of therapy by greater than seven days because of hematologic toxicity.

7.3 Definition of Maximum Tolerated Dose (MTD)

For the purposes of this study, the MTD will be exceeded by the dose at which two out of at most six patients experience dose-limiting toxicity (DLT). The MTD will be equivalent to the recommended Phase II schedule.

7.4 Definition of Molecular Response

Response to therapy will be evaluated both at the clinical and molecular level. Molecular response will be determined by evaluation of tumor material for evidence of the phenotypic effect of anti-erbB-2 sFv expression in target cells. These effects include: 1) evidence of transfection of targeted ovarian cancer cells with sFv construct, 2) down regulation of cell surface erbB-2 expression, and 3) inhibition of cellular proliferation. These assays will be carried out in primary tumor material exactly as previously described for cancer cell lines in the preliminary data section.

7.5 Clinical Response Criteria

Clinical response is not a primary endpoint of this study, but any responses will be documented and reported. Best response achieved during the treatment period will be used and reported as follows:

7.51 Complete Response: Disappearance of all clinical and laboratory signs and symptoms of active disease for a minimum of six weeks.

7.52 Partial Response: A 50% or greater reduction in the size of the lesions as defined by the sum of the products of the longest perpendicular diameters of all measured lesions lasting for a minimum of four weeks. No lesions may increase in size and no new lesions may appear.

7.53 Minor Response: A 25 to 49% reduction in the sum of the products of the longest perpendicular diameters of all measured lesions lasting for a minimum of one month. No lesion may increase in size and no new lesions may appear. This also includes patients with evaluable but non-measurable disease and objective but incomplete response.

7.54 Stable Disease: Tumor measurements not satisfying the criteria for response or progression.

7.55 Progressive Disease: An increase of 25% or more in the sum of the products of the longest perpendicular diameters of all measured indicator lesions compared to the smallest previous measurement or the appearance of a new lesion.

8.0 Study Monitoring and Reporting Procedures

8.1 A copy of the protocol, informed consent, and IRB approval will be on file in the office of the principal investigators.

8.2 Clinicopathologic data including adverse reactions will be recorded on standardized data forms.

8.3 Adverse Effects Reporting Procedure

8.31 The adverse reaction should be reported to the principle investigators. The following guidelines must be followed: (NCI—The requirement for prompt reporting of adverse events to Investigational Drug Branch by phone within 24 hours, followed by a written report within 10 working days.)

All life-threatening events (Grade 4) which may be due to drug administration.

All fatal events.

The first occurrence of any previously unknown clinical event (regardless of grade).

8.32 Written report to follow within 10 working days to:
Investigational Drug Branch
P.O. Box 30012
Bethesda, MD 20824

9.0 Statistical Considerations

This is a Phase I study. The primary objective is to determine the maximally tolerated dose (MTD) of a recombinant adenovirus containing an anti-erbB-2 sFv antibody gene. A commonly used design of Phase I trials is adopted for this study as described in section 5.0. Descriptive statistics will be used to summarize the results. No other statistical methods will be used for this Phase I study.

A total of three dose levels is planned for this study. Assuming on average 4.5 patients per each dose level (some may require 3 patients and some may require 6 patients), approximately 14 patients are required. The actual number of patients may be smaller if the study stops early.

Projected total number of patients: 14
Maximal total number of patients: 18
Projected annual accrual: 10 patients
Projected study duration: 2 years

10.0 Bibliography

1. Boring, C., Squires, T., Tong, T. and Montgomery, S. Cancer statistics, 1994. *CA—A Cancer Journal for Clinicians* **44,** 7–26 (1994).
2. Bloss, J.D., Liao, S.-Y., Buller, R.E., Manetta, A., Berman, M.L., McMeekin, S., Bloss, L.P. and DiSaia, P.J. Extraovarian peritoneal serous papillary adenocarcinoma: A case-control retrospective comparison to papillary adenocarcinoma of the ovary. *Gynecol Oncol* **50,** 347–351 (1993).
3. Ozols, R. and Young, R. Chemotherapy of ovarian cancer. *Semin Oncol* **11,** 251 (1984).
4. Omura, G., Blessing, J., Ehrlich, C., Miller, A., Yordan, E., Creasman, W. and Homesley, H. A randomized trial of cyclophosphamide and doxorubicin with or without cisplatin in advanced ovarian carcinoma: A Gynecologic Oncology Group study. *Cancer* **57,** 1725–1730 (1986).
5. Neijt, J.P., van der Burg, M.E.L., Vriesendorp, R., van Lindert, A.C.M., van Lent, M. Ten Bokkel Huinink, W.W., van Oosterom, A.T., Kooyman, C.D., Hamerlynck, J.V.T.H., van Houwelingen, J.C. and Pinedo, H.M. Randomized trial comparing combination chemotherapy regimens (Hexa-CAF vs CHAP-5) in advanced ovarian carcinoma. *Lancet* **2,** 594–600 (1984).
6. Decker, D., Fleming, T., Malkasian, G., Webb, M., Jeffries, J. and Edmonson, J. Cyclophosphamide plus cis-platinum in combination: Treatment program for stage III or IV ovarian carcinoma. *Obstet Gynecol* **60,** 481 (1982).
7. McGuire, W., Hoskins, W., Brady, M., Kucera, P.H., Look, K.Y., Partridge, E.E. and Davidson, M. A phase III trial comparing cisplatin/cytoxan (CC) and cisplatin/taxol (PT) in advanced ovarian cancer (AOC). *Proceedings Am Soc Clin Oncol* **12**(Abstract 808), 255 (1993).
8. McGuire, W.P. Taxol: a new drug with significant activity as a salvage therapy in advanced epithelial ovarian carcinoma. *Gynecol Oncol* **51**(1), 78–85 (1993).
9. Vergote, I., Himmelman, A., Frankendal, B., Scheistroen, M., Vlachos, K. and Trope, C. Hexamethylmelamine as second-line therapy in platin-resistant ovarian cancer. *Gynecol Oncol* **47,** 282–286 (1992).
10. Sutton, G.P., Blessing, J.A., Homesly, H.D., Berman, M.L. and Malfetano, J. Phase II trail of ilosfamide and mesna in advanced epithelial ovarian carcinoma: A Gynecologic Oncology Group study. *J Clin Oncol* **7,** 1672–1676 (1989).
11. Markman, M. Current status of intraperitoneal therapy for ovarian cancer. *Curr Opinion Obstet Gynecol* **5,** 99–104 (1993).
12. Thomas, G.M. and Dembo, A.J. Integrating radiation therapy into the management of ovarian cancer. *Cancer* **71,** 1710–1718 (1993).
13. Rosenberg, S.A., Anderson, W.F., Blaese, M., Hwu, P., Yannelli, J.R., Yang, J.C., Topalian, S.L., Schwartzentruber, D.J., Weber, J.S., Ettinghausen, S.E., Parkinson, D.N. and White, D.E. The development of gene therapy for the treatment of cancer. *Annals of Surgery* **218,** 455–464 (1993).
14. Karp, J.E. and Broder, S. New directions in molecular medicine. *Cancer Research* **54,** 653–665 (1994).
15. Gutierrez, A.A., Lemoine, N.R. and Sikora, K. Gene therapy for cancer. *The Lancet* **339,** 715–721 (1992).
16. Lemoine, N. and Sikora, K. Interventional genetics and cancer treatment. *BMJ* **306,** 665–666 (1993).
17. Vanchieri, C., Opportunities "opening up" for gene therapy. *JNCI* **85,** 90–91 (1993).
18. Freeman, S.M. and Zwiebel, J.A. Gene therapy of cancer. *Cancer Investigation* **11,** 676–688 (1993).
19. Dorudi, S., Northover, J.M.A. and Vile, R.G. Gene transfer therapy in cancer. *Br J Surg* **80,** 566–572 (1993).
20. Sikora, K. Gene therapy for cancer. *Tibtech* **11,** 197–201 (1993).
21. Krol, A.V., Mol, J.N.M. and Stuitje, A.R. Modulation of eukaryoutic gene expression by complement RNA or DNA sequences. *Biotechniques* **6,** 958–976 (1988).
22. Helene, C. and Toulme, J.-J. Specific regulation of gene expression by antisense, sense and antigene nucleic acids. *Biochemica et Biophysica* **1049,** 99–125 (1990).
23. Zon, G. Oligonucleotide as potential chemotherapeutic agents. *Pharmaceutical Research* **5,** 539–549 (1988).
24. Stein, C.A. and Cheng, Y.-C. Antisense oligonucleotides as therapeutic agents—Is the bullet really magic? *Science* **261,** 1004–1012 (1993).
25. Kashani-Sabet, M., Funato, T., Florenes, V.A., Fodstad, O. and Scanlon, K.J. Suppression of a neoplastic phenotype in vivo by an anti-ras ribozyme. *Cancer Research* **54,** 900–902 (1994).
26. Wickstrom, E.L., Bacon, T.A., Gonzalez, A., Freeman, D.L., Lyman, G.H. and Wickstrom, E. Human promelocytic leukemia HL-60 cell proliferation and c-myc protein expression are inhibited by antisense pentadecadeoxynucleotide targeted against c-myc mRNA. *Proc Natl Acad Sci USA* **85,** 1028–1032 (1988).
27. Georges, R.N., Mukhopadhyay, T., Zhang, Y., Yen, N. and Roth,

J.A. Prevention of orthotopic human lung cancer growth by intratrachela instillation of a retroviral antisense k-ras construct. *Cancer Research* **53,** 1743–1746 (1993).
28. Ratahczak, M.A., Kant, J.A., Luger, S.M., Hijiya, N., Zhang, J., Zon, G. and Gerwitz, A.M. In vivo treatment of human leukemia in a scid mouse model with c-myb antisense oligonucleotides. *Proc Natl Acad Sci USA* **89,** 11823–11827 (1992).
29. Iverson, P.L., Zhu, S., Meyer, A. and Zon, G. Cellular uptake and subcellular distribution of phosphorothioate into cultured cells. *Antisense Research and Development* **2,** 211–222 (1992).
30. Bergan, R., Connell, Y., Fahmy, B. and Neckers, L. Electroporation enhance c-myc antisense oligodeoxynucleotide efficacy. *Nucleic Acids Research* **21,** 3567–3573 (1993).
31. Citro, G., Perrotti, D., Cucco, C., D'Agnano, I., Sacchi, A., Zupi, G. and Calabretta, B. Inhibition of leukemia cell proliferation by receptor-mediated uptake of c-myb antisense oligodeoxynucleotides. *Proc Natl Acad Sci USA* **89,** 7031–7035 (1992).
32. Seemayer, T.A. and Cavenee, W.K. Biology of disease—Molecular mechanisms of oncogenesis. *Laboratory Investigation* **60,** 585–599 (1989).
33. Duke, R.C. and Cohen, J.J. Morphological and biochemical assays of apoptosis. *Current Protocols Supplement* **3** (1992).
34. Bertram, J., Killian, M., Brysch, W., Schlingensiepen, K.-H. and Kneba, M. Reduction of erbB2 gene product in mammary carcinoma cell lines by erbB2 mRNA-specific and tyrosine kinase consensus phosphorothioate antisense oligonucleotides. *Biochemical and Biophysical Research Communications* **200,** 661–667 (1994).
35. Brysch, W., Magal, E., Louis, J.-C., Knust, M., Klinger, I. and Schlinggensiepen, R. Inhibition of p185c-erbB-2 proto-oncogene expression by antisense oligodeoxynucleotides down-regulates p185-associated tyrosine-kinase activity and strongly inhibits mammary tumor-cell proliferation. *Cancer Gene Therapy* **1,** 99–105 (1994).
36. Vaux, D.L. Toward an understanding of the molecular mechanisms of physiologic cell death. *Proc Natl Acad Sci USA* **90,** 786–789 (1993).
37. Williams, G.T. Programmed cell death: apoptosis and oncogenesis. *Cell* **65,** 1097–1098 (1991).
38. Mishell, B.B., Shiigi, S.M., Henry, C., Chan, E.L., North, J., Gaillily, R., Slomich, M., Miller, K., Marbrook, J., Parks, D. and Good, A.H. Preparation of mouse cell suspensions, *Selected Methods in Cellular Immunology* 21–22 (1980).
39. Deshane, J., Loechel, F., Conry, R.M., Siegal, G.P., King, C.R. and Curiel, D.T. Intracellular single-chain antibody directed against erbB2 down regulates cell surface erbB2 and exhibits a selective anti-proliferative effect in erbB2 overexpressing cancer cell lines. *Gene Therapy* **1,** 332–337 (1994).
40. Setoguchi, Y., Jaffe, H.A., Chu, C.-S. and Crystal, R.G. Intraperitoneal in vivo gene therapy to deliver alpha1-antitrypsin to the systemic circulation. *Am J Respir Cell Mol Biol* **10,** 369–377 (1994).
41. Smythe, W.R., Kaiser, L.R., Hwang, H.C., Amin, K.M., Pilewski, J.M., Eck, S.J., Wilson, J.M. and Abelda, S.M. Successful adenovirus-mediated gene transfer in an in vivo model of human malignant mesothelioma. *Ann Thorac Surg* **57,** 1395–1401 (1994).
42. Yarden, Y. and Ullrich, A. Growth factor receptor tyrosine kinases. *Annu Rev Biochem* **57,** 443–478 (1988).
43. Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G. and Keith, D.E. Studies of the HER-2/neu proto-oncogene in the human breast and ovarian cancer. *Reports,* 707–712 (1989).
44. Muller, W.J., Sinn, E., Pattengale, P.K., Wallace, R. and Leder, P. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell* **54,** 105–115 (1988).
45. Hudziak, R.M., Schlessinger, J. and Ulrich, A. Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells. *Proc Natl Acad Sci USA* **84,** 7159–7163 (1987).
46. Giovanella, B.C., Vardeman, D.M., Williams, L.J., Taylor, D.J., Deipolyi, P.D., Greef, P.J., Stehlin, J.S., Ullrich, A., Cailleau, R., Slamon, D.J. and Gary, H.E. Heterotransplantation of human breast carcinomas in nude mice. Correlation between successful heterotransplants, poor prognosis and amplification of HER-2/Neu oncogene. *Int J Cancer* **47,** 66–71 (1991).
47. Hynes, N.E. Amplification and overexpression of the erbB-2 gene in human tumors: Its involvement in tumor development, significance as a prognostic factor, and potential as a target for cancer therapy. *Cancer Biology* **4,** 19–26 (1993).
48. Braly, P., Doroshow, J. and Hoff, S. Technical aspects of intraperitoneal chemotherapy in abdominal carcinomatosis. *Gynecol Oncol* **25,** 319–333 (1986).
49. Jenkins, J., Sugarbaker, P.H., Gianla, F.J. and Myers, C.E. Technical considerations in the use of intraperitoneal chemotherapy administered by Tenckhoff catheter. *Surg Gynecol Obstet* **154,** 858–864 (1982).

Figure 19. Large scale viral preparation of adenovirus. Grow 293 to ~80% confluence in 75-cm flasks × 20 in DMEM/10% FCS/PCN + Strep

↓

Aspirate off media

↓

Dilute viral stock in 100 ml of DMEM/2% heat-inactivated FCS and add 5 ml/flask

↓

Incubate 37°C, 5% CO_2, 1–2 hr

↓

Add 10 ml of complete media/flask (DMEM/10% FCS/PCN + Strep)

↓

Incubate as above 48–72 hr until prominent cytopathic effect seen in cells

↓

Harvest cells + media from flasks in 50-ml capped centrifuge tubes

↓

Centrifuge 1,000 rpm, 10 min, 4°C Beckman GS-GR Centrifuge ("Tabletop")

↓

Aspirate off media and resuspend cell pellets in total volume of 10 ml of 2% FCS media

↓

Freeze-thaw × 4

↓

Centrifuge as above except at 4,000 rpm, 20 min, 4°C and harvest supernatant; save at 4°C while setting up gradients

↓

Set up CsCl gradient tubes as follows:

SW 28 tube: Step 20 ml of 1.33 CsCl
10 ml of 1.45 CsCl
5 ml of viral cell lysate
centrifuge 18,000 rpm, $1^1/_2$ hr, 20°C

↓

Harvest lowest band w/syringe (in approximately 2 ml)

Dilute 1:1 with 5 m*M* HEPES, pH 7.8
↓
SW41 tube: 4 ml of 1.33 CsCl
4 ml of 1.45 CsCl
4 ml of virus from 1st spin
↓
Centrifuge 25,000 rpm, o/n (>18 hr), 20°C
↓
Harvest lower band
↓
Use virus directly for chemical coupling to poly-L-lysine or preserve as follows:
dilute: 1 part virus
5 parts viral preservation media
Store in aliquots at −70°C

Recipes

Viral preservation media:
1 *M* Tris, pH 8.0—1 ml
5 *M* NaCl—2 ml
0.1 gm BSA
bring volume up to 50 ml w/H_2O
add 50 ml glycerol
filter sterilize and store at 4°C
CsCl 1.33 454.2 mg CsCl/ml
20 m*M* HEPES, pH 7.4
CsCl 1.45 609.0 mg CsCl/ml
20 m*M* HEPES, pH 7.4

Notes:
1) Perform all steps in a sterile manner.
2) P2 level of containment.

Patient Informed Consent

PHASE I STUDY OF RECOMBINANT ADENOVIRUS VECTOR-MEDIATED DELIVERY OF AN ANTI-ERBB-2 SINGLE-CHAIN ANTIBODY GENE FOR PREVIOUSLY TREATED OVARIAN AND EXTRAOVARIAN PATIENTS

INTRODUCTION AND PURPOSE

I understand that I have a form of ovarian or extraovarian cancer that has been resistant to standard chemotherapy or has recurred after an initial response to standard chemotherapy. Investigators at The University of Alabama at Birmingham Cancer Center are currently looking into experimental treatments to improve therapy for patients with previously treated persistent or recurrent ovarian and extraovarian cancer. I have been asked by my physician to participate in a research study using a form of gene therapy. I have also been told that I have the option not to participate. I understand that I can take as much time as I would like to decide whether or not to participate in this study.

The purpose of this study is to determine the maximally tolerated dose and to evaluate the effectiveness and safety of a particular form of gene therapy in the treatment of ovarian cancer. I understand that this form of gene therapy involves injecting a special form of genetic material into my abdominal cavity. The material that will be injected is a gene which produces an antibody. The antibody is used to block another gene (the erbB-2 gene) that is found in cancer cells. It is hoped that the anti-erbB-2 antibody gene will be well tolerated and will prevent the expression of the erbB-2 gene, thus inhibiting the growth of my cancer.

The anti-erbB-2 gene will be delivered into my tumor cells by a virus. The virus, called adenovirus, is a cold virus that has been modified so that it does not cause disease but will easily enter the tumor cells. This adenovirus has been used to carry other genetic material and has been administered to a number of patients by a variety of routes in several other gene therapy protocols with no unexpected effects noted. This trial will evaluate the physical side effects as well as the response of my tumor cells to the injection of this adenovirus containing an anti-erbB-2 antibody gene.

EXPLANATION OF PROCEDURES

I understand that the procedure will involve receiving a single dose of the adenovirus containing the anti-erbB-2 antibody gene. This material will be administered into my abdominal cavity through a catheter or tube. I understand that placement of the catheter is a surgical procedure.

The purpose of this study is to determine the highest dose of the adenovirus containing the anti-erbB-2 antibody gene that humans can tolerate without significant side effects. Therefore, the dose will be increased in successive groups of patients. The dose I receive will depend on the experience of prior patients in this study. Depending on the progress of the study, I will receive one of three possible dose levels. I understand that I will receive this treatment in the hospital and will remain hospitalized for 24 hours following treatment for observation.

I understand that prior to the study, I will undergo a physical exam, an HIV test, blood tests, aspiration of fluid from my abdominal cavity through the catheter and a CT scan of my abdomen and pelvis.

If I am eligible and choose to participate in this study, I understand that I will be hospitalized to receive a single treatment of the assigned dose of adenovirus containing the anti-erbB-2 antibody gene through the catheter in my abdominal cavity. I will remain in the hospital for 24 hours following this treatment. Once a week for 8 weeks following my treatment, I will have a physical exam, blood tests, and aspiration of the fluid in my abdominal cavity through the catheter. I will have a CT scan of my abdomen and pelvis eight weeks after treatment.

Following the eighth week, I will be scheduled for periodic clinic visits to assess my condition and to evaluate long-term side effects. I understand that I will be followed by the physicians involved in this study for the rest of my life and that I should keep these physicians informed of my current address. I further understand that a member of my family or someone else appointed by me should inform the physicians involved in

the study of my medical status in the event that I cannot attend scheduled follow-up visits.

I understand that, should death occur during the course of this study or thereafter from a cause unrelated to my cancer, an autopsy may be requested by the physicians involved in this study. I further understand that I should inform the physicians if I am willing to give consent for this autopsy and to make my wishes concerning autopsy known to my family.

RISKS AND DISCOMFORTS

I understand that possible risks associated with surgical placement of a catheter into my abdomen include bleeding, infection or injury to abdominal organs.

I understand that this form of gene therapy has not been given to human patients. Animal studies have noted no toxicity or side effects. The potential acute or long-term side effects of having this gene taken into my cells are unknown. Despite extensive efforts to assure its safety, I understand that other unforeseen problems may arise including the very remote possibility of death. I understand that I will be carefully followed and evaluated for any side effects.

MANAGEMENT OF SIDE EFFECTS

I understand that care will be taken to keep complications at a minimum. Should any of the previously mentioned side effects or others occur, I will be evaluated by the physicians involved in this study or their associates to determine what steps may be taken to reduce or eliminate them (for example, interrupt therapy or prescribe medication to control side effects).

ALTERNATIVE TREATMENTS

I understand that there are alternative treatments to this protocol which may include additional chemotherapy or other experimental programs. I also understand that I may elect to have no further therapy and to receive supportive care only. These alternatives have been discussed with me by the physicians involved in this study prior to my enrollment in this study.

POTENTIAL BENEFITS

I understand that it is not possible to predict whether any personal benefit will result from participation in this study. I understand that by participating in this clinical trial, I am contributing to science which will benefit future cancer patients by determining if this treatment is tolerated and effective against my type of cancer.

CONFIDENTIALITY

I understand that all or part of my medical records will be reviewed and analyzed by physicians, statisticians and other study personnel, along with the records of all other patients participating in this study from UAB. I understand that my hospital records, doctor's office records, laboratory, operating room and other records may be audited by representatives of the NCI, the Food and Drug Administration (FDA) or other responsible organizations/agencies that may be concerned about the content or character of my records. I understand that the usual medical records precautions will be taken to otherwise maintain the privacy and confidentiality of my records. The information gained from this study will be used to evaluate the treatment described and will appear in medical journals as scientific papers. I understand that my name will not appear in any such report. I understand that other media interests may report the results of this study. Every effort will be taken to maintain the confidentiality of my records, and my name will not appear in any such report.

WITHDRAWAL FROM STUDY

I understand that I am free to withdraw my consent to participation in this treatment at any time without penalty or prejudice against future medical care I may receive at this institution. I understand that my physician may remove me from the study if needed to guarantee that my best interests are served. I understand that I may choose to not continue with further treatment or evaluation at any time during the study. I also understand that should I withdraw from this study, I should maintain contact with the physicians involved in case I should have any adverse effects from treatment.

I have been assured that any new information regarding this study and any treatment it involves which might affect my willingness to continue participation will be related to me in an appropriate and timely manner.

COMPENSATION

I understand that I will receive *NO* payment or compensation of any kind in exchange for my participation in this study. I understand that the anti-erbB-2 antibody gene will be provided free of charge. All other reasonable and customary charges associated with treatment of persistent/recurrent ovarian or extraovarian cancer will be my responsibility or my insurance company's responsibility. I further understand that UAB, the FDA, and the NCI have made *NO* provision for payment to me in the event of physical or psychological injury resulting from the research procedures. In the event such injury occurs, medical treatment at this institution is provided, but is not provided free of charge. Third party carriers such as private insurance companies, Medicare or Medicaid, may or may not agree to pay for medical care required as a result of investigational cancer treatment. If these providers refuse to pay for such services, I understand that I am liable for any hospital or other medical bill. We will intervene on your behalf, if at all possible, to clarify whether your medical expenses will be covered by your insurance company. Additionally, our social worker Mrs. Michael K. Schepps, can be reached at 934-4737 should you wish to inquire about ob-

taining coverage for your medical expenses prior to making a decision as to whether or not you wish to participate.

QUESTIONS

I have the right to ask questions and receive reasonable answers to my satisfaction at any time, now or later. I am encouraged to seek information from agencies such as the American Cancer Society or the Cancer Information Service that might help my understanding of my disease and treatment. I can contact Dr. Ronald D. Alvarez, his associates and nurses at (205) 934-4986 or Dr. David T. Curiel at (205) 934-8627 for any questions or problems that arise.

AGREEMENT

I understand that I am medically and physically qualified to participate in this study. I am making a decision whether or not to participate. The nature of this research project has been fully explained to me and I understand my options for treatment at this time. I understand that I am not waiving any of my legal rights by signing this document. I have received a copy of this written informed consent, in addition to a full verbal explanation. My signature below indicates that I agree to participate in this study given the information provided.

Signature of Patient	Date
Signature of Witness	Date
Signature of PI or Attending Physician	Date

AUTHORIZATION TO RELEASE MEDICAL INFORMATION

Date _____

Permission is hereby given _____
to release my medical information to:

Ronald D. Alvarez, M.D. & Associates
Department of Obstetrics & Gynecology
Division of Gynecologic Oncology
The University of Alabama at Birmingham
618 South 20th Street
Birmingham, Alabama 35233-7333

Signed _____
Patient

Signed _____
Witness

Identifying information at time patient was seen:

Name _____

Address _____

Telephone Number(s) _____

Birth Date _____ Race _____

Social Security Number _____

Schema

Establish maximal tolerated single dose (MTD) of intraperitoneal recombinant adenovirus encoding anti-erbB-2 antibody (sFv)

	Intraperitoneal PFU	*Evaluation*
Recurrent ovarian/ extraovarian cancer confined to abdomen	$\rightarrow$ 10^8 10^{10} 10^{12}	$\rightarrow$ Toxicity Safety Molecular response